

FIG. 1

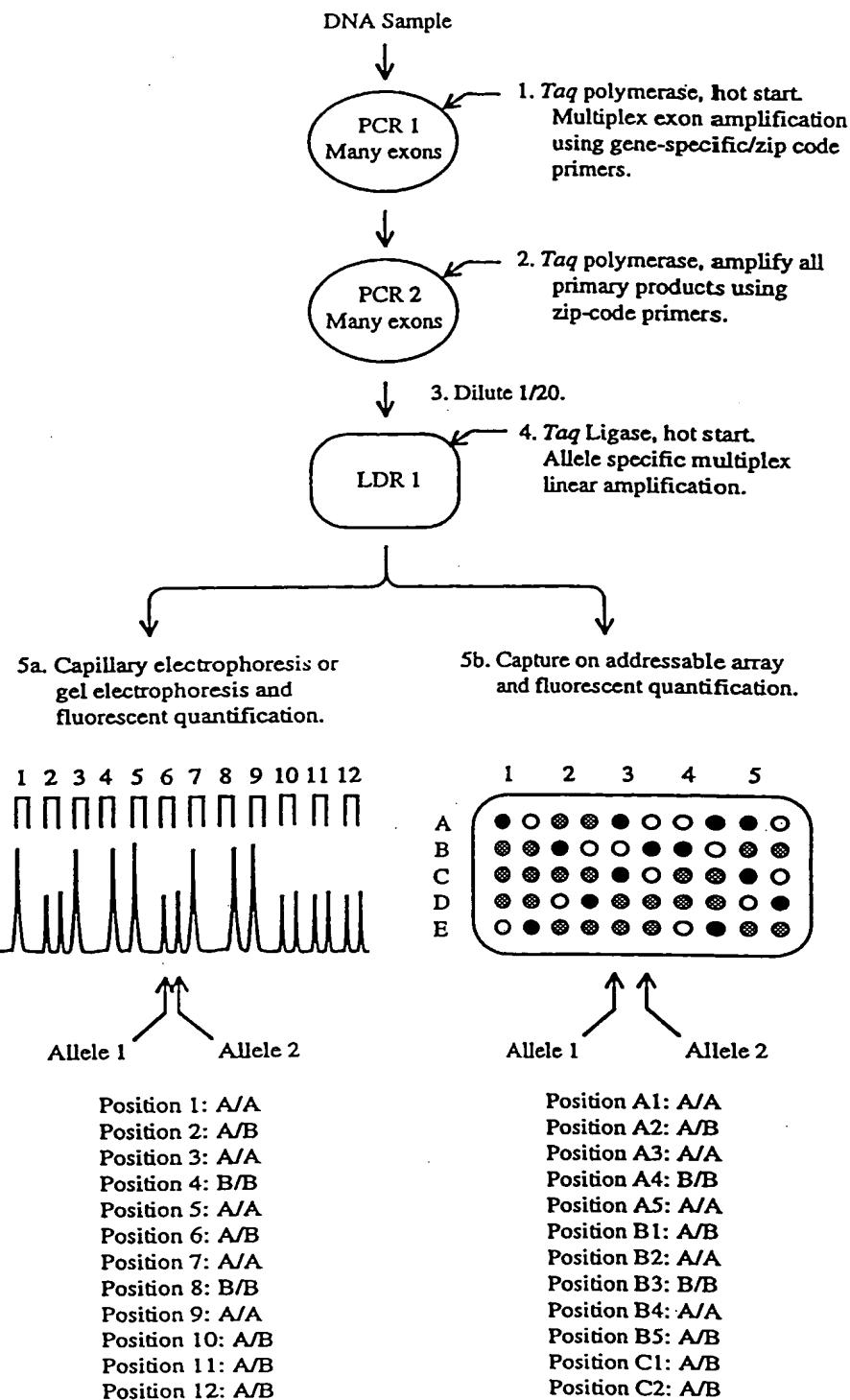
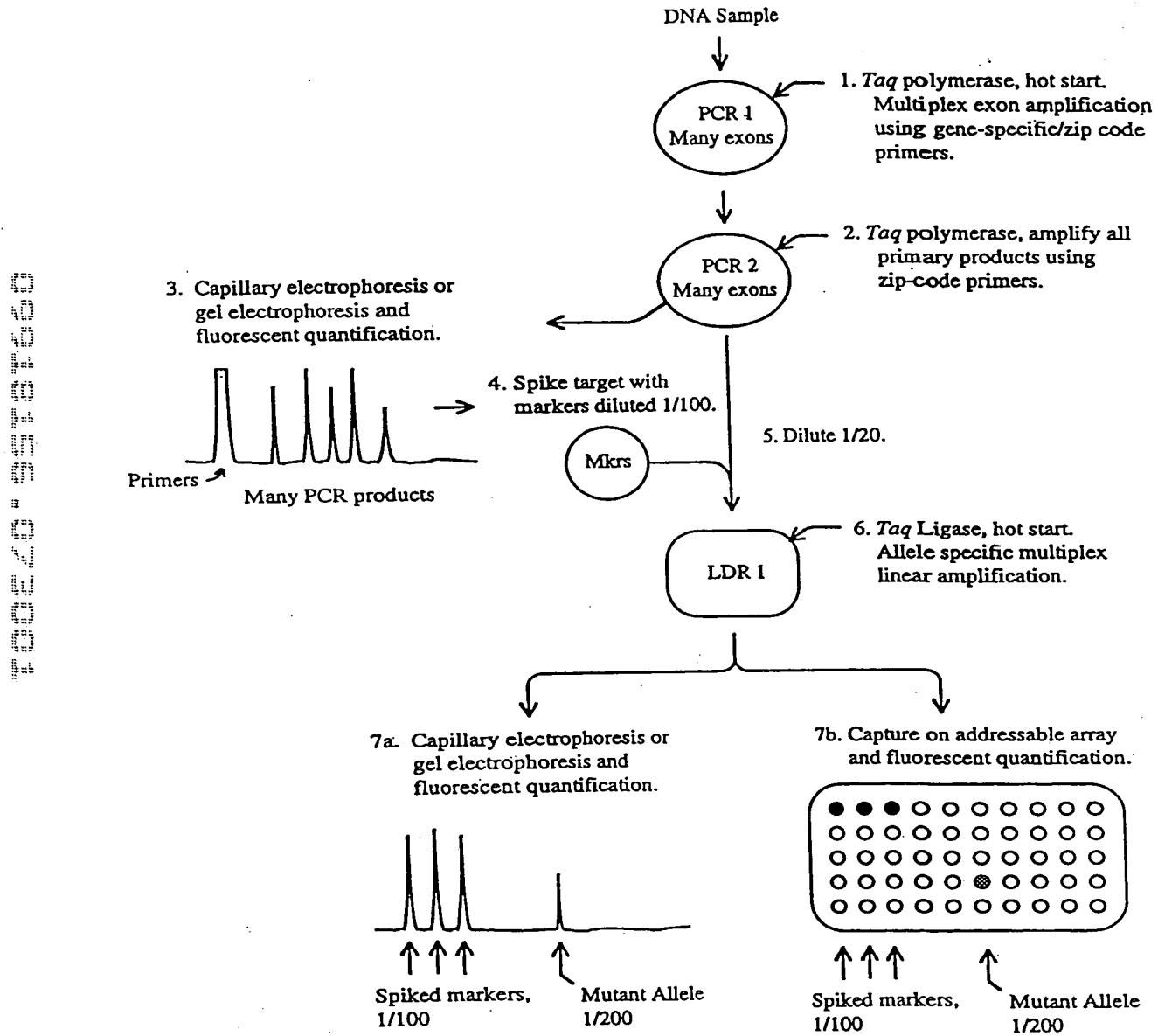
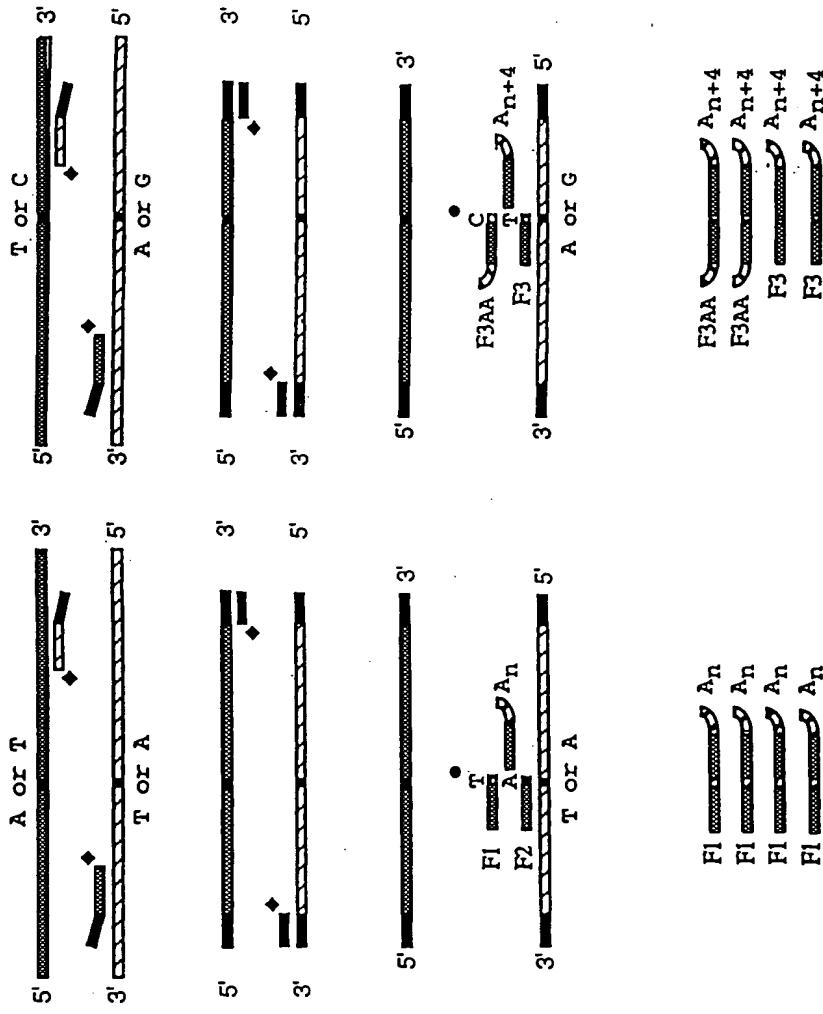


FIG. 2

**FIG. 3**

PCR/ PCR/ LDR

1. PCR amplify regions containing allelic variations using gene-specific/zip code primers, dNTPs and Taq polymerase. ♦
2. PCR amplify all primary products using zip code primers, dNTPs and Taq polymerase.
3. Perform LDR using allele-specific LDR primers and thermostable ligase. ● Allele-specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.



4. Separate fluorescent products on a DNA sequencer and quantify each allele.

Homozygous: T allele only.

Heterozygous: C and T alleles.

FIG. 4

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PCR/ PCRLDR

1. PCR amplify regions containing allelic variations using gene-specific zip code primers, dNTPs and *Taq* polymerase. ♦
2. PCR amplify all primary products using zip code primers, dNTPs and *Taq* polymerase. ♦
3. Perform LDR using allele-specific LDR primers and thermostable ligase. ● Allele-specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.
4. Separate fluorescent products on a DNA sequencer and quantify each allele.

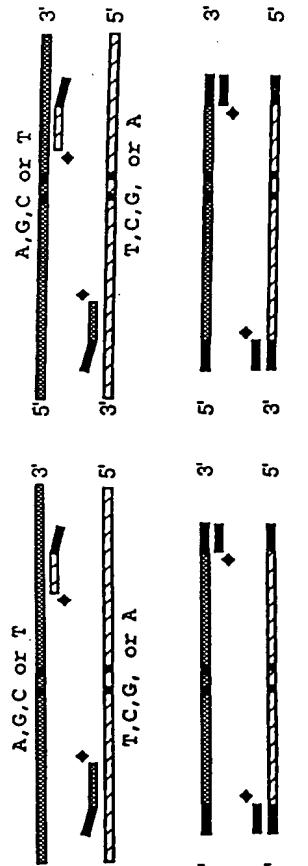
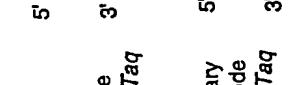
Heterozygous: G and C alleles.

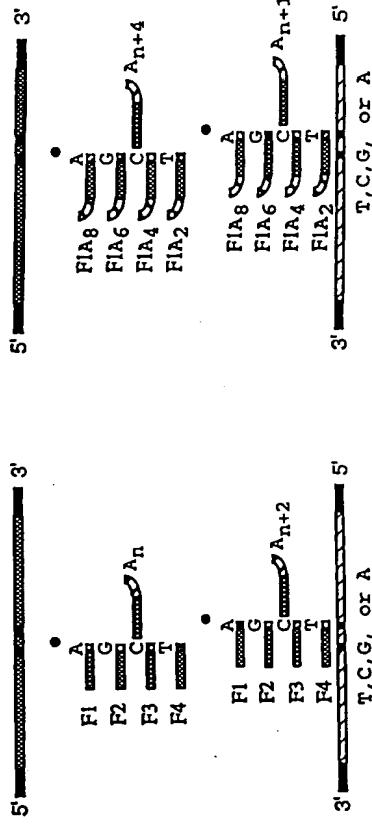
Heterozygous: A and C alleles.

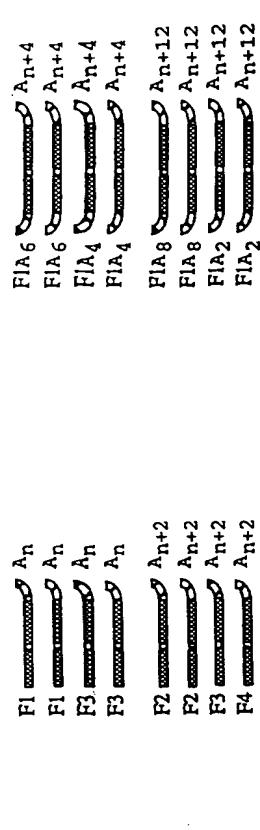
FIG. 5

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PCR/ PCR/LDR : Nearby alleles

1. PCR amplify regions containing allelic variations using gene-specific/zip code primers, dNTPs and *Taq* polymerase. ♦
 
2. PCR amplify all primary products using zip code primers, dNTPs and *Taq* polymerase.
 

3. Perform LDR using allele-specific LDR primers and thermostable ligase.
 - Allele-specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.

4. Separate fluorescent products on a DNA sequencer and quantify each allele.
 

1st Position.
Heterozygous: A and C alleles.
2nd Position.
Heterozygous: G, C and T alleles.

FIG. 6

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PCR/ PCR/LDR : Adjacent alleles, cancer detection

1. PCR amplify regions containing allelic variations using gene-specific/zip code primers, dNTPs and *Taq* polymerase. ♦

2. PCR amplify all primary products using zip code primers, dNTPs and *Taq* polymerase.

3. Perform LDR using allele-specific LDR primers and thermostable ligase. Allele-specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.

4. Separate fluorescent products on a DNA sequencer and quantify each allele.

FIG. 7

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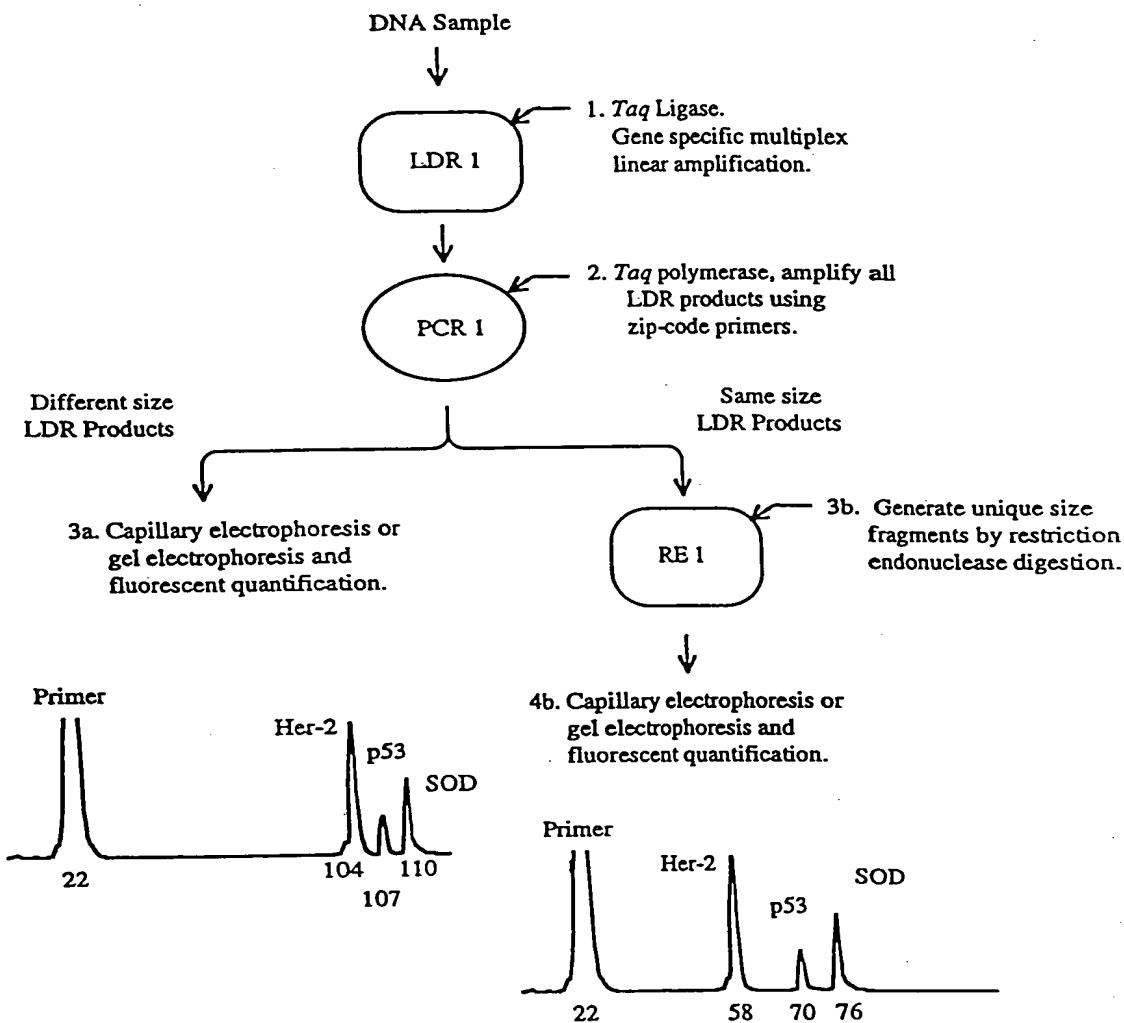
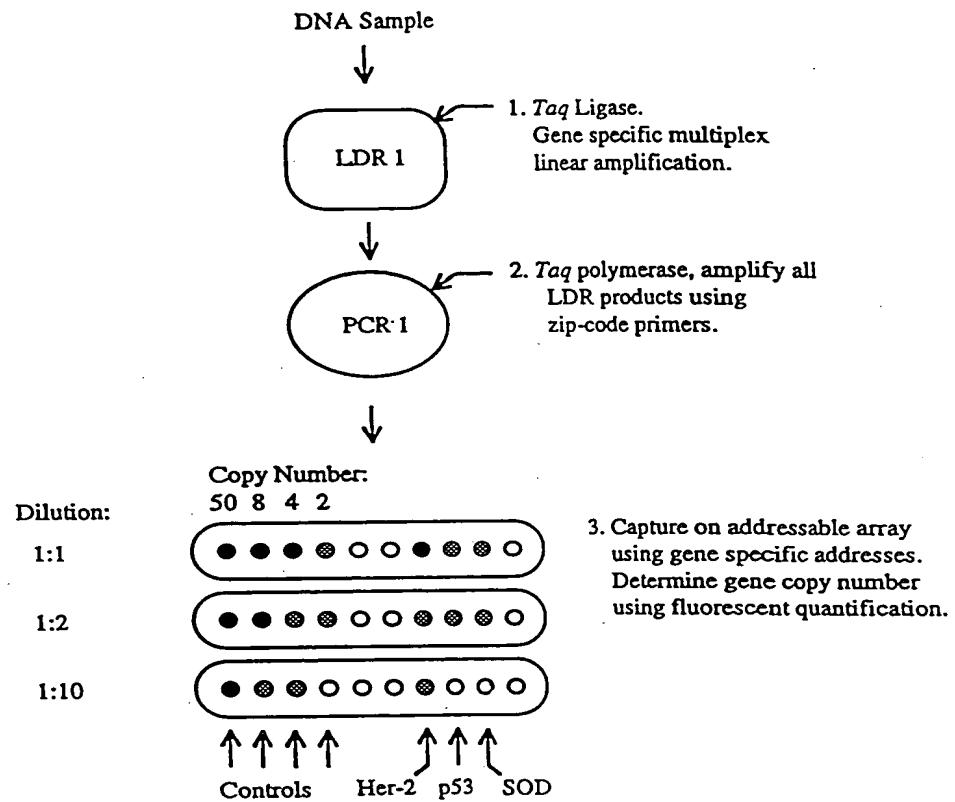
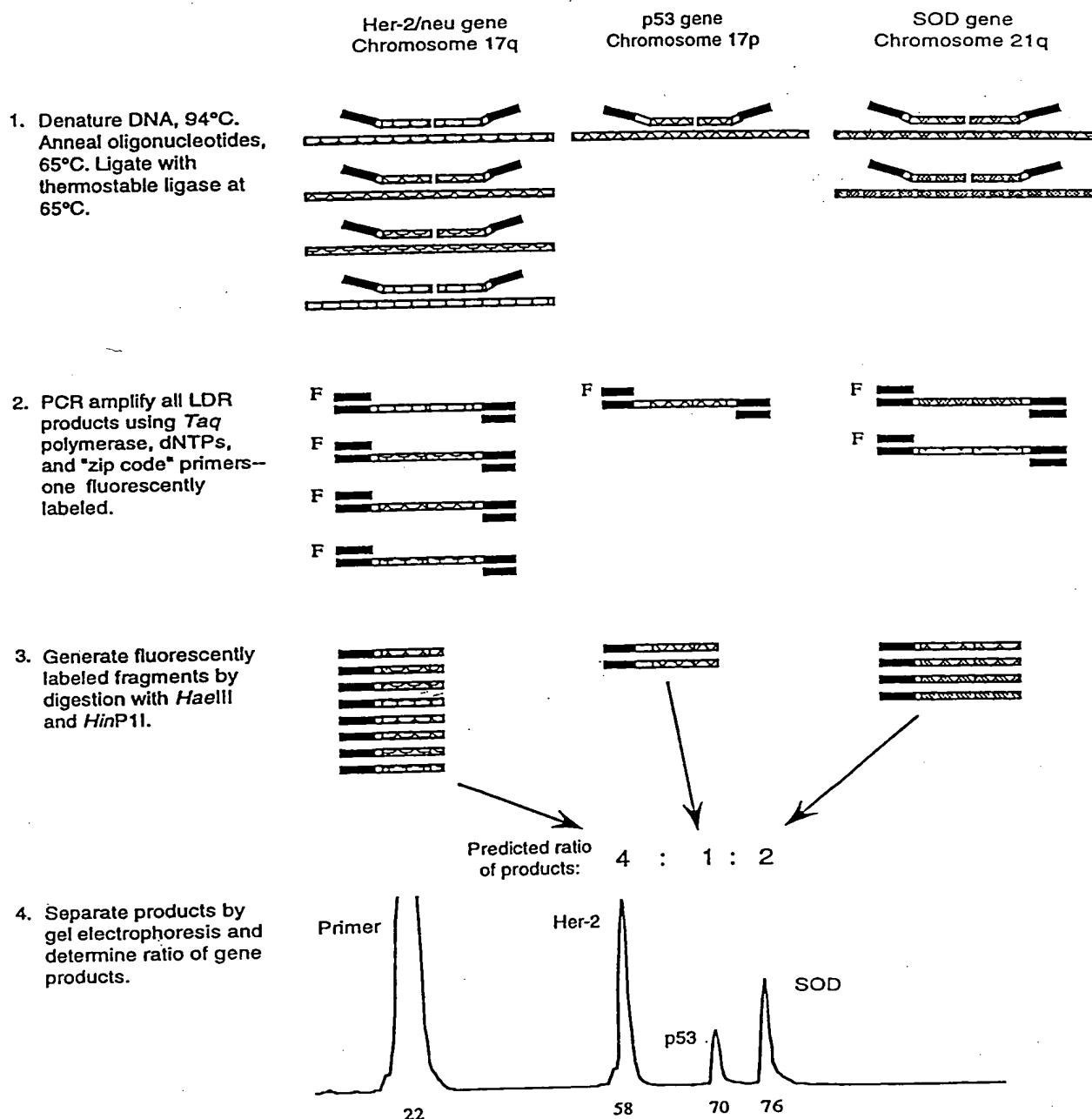


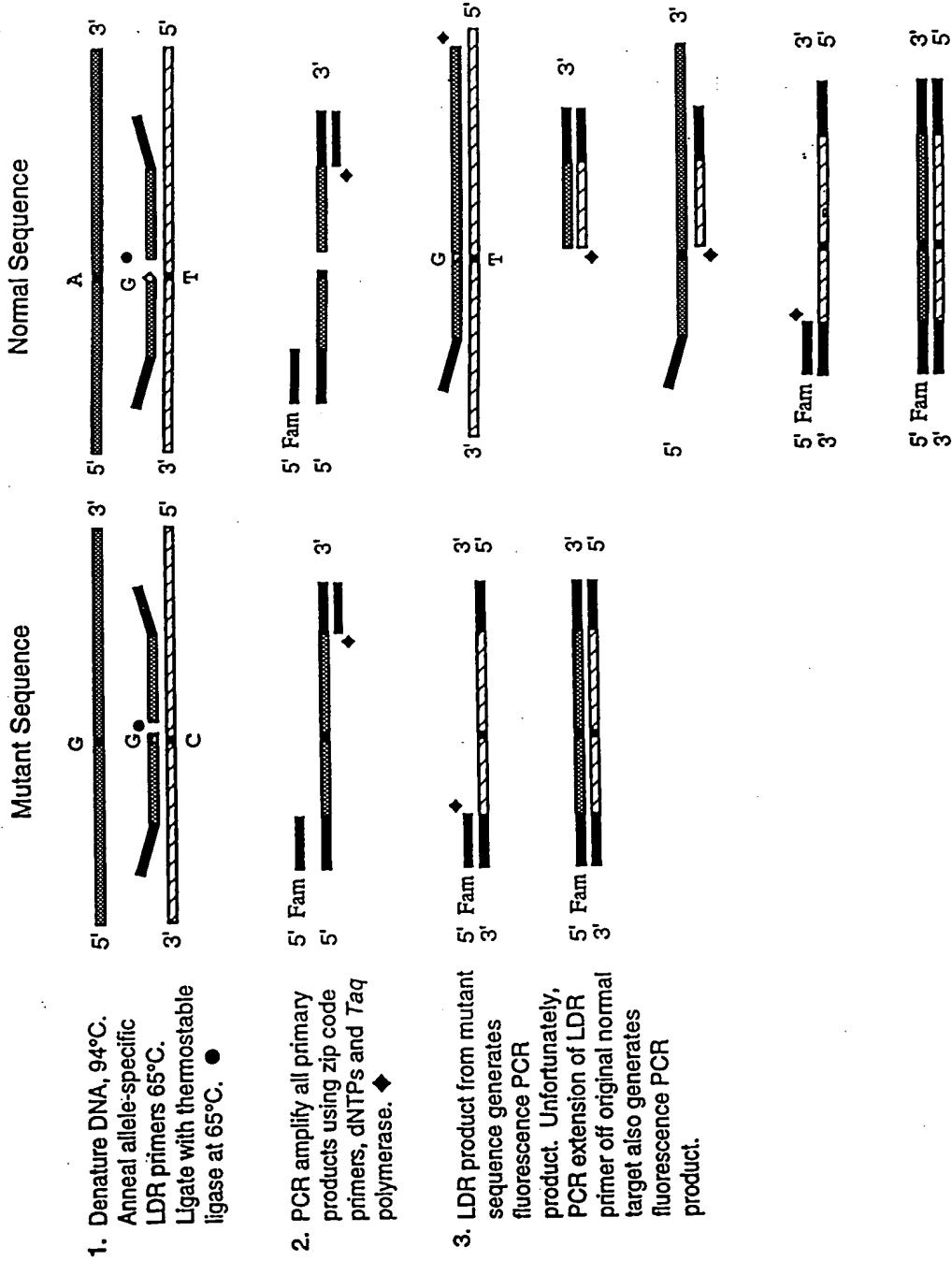
FIG. 8

**FIG. 9**

LDR / PCR: Multiplex detection of gene amplifications and deletions

**FIG. 10**

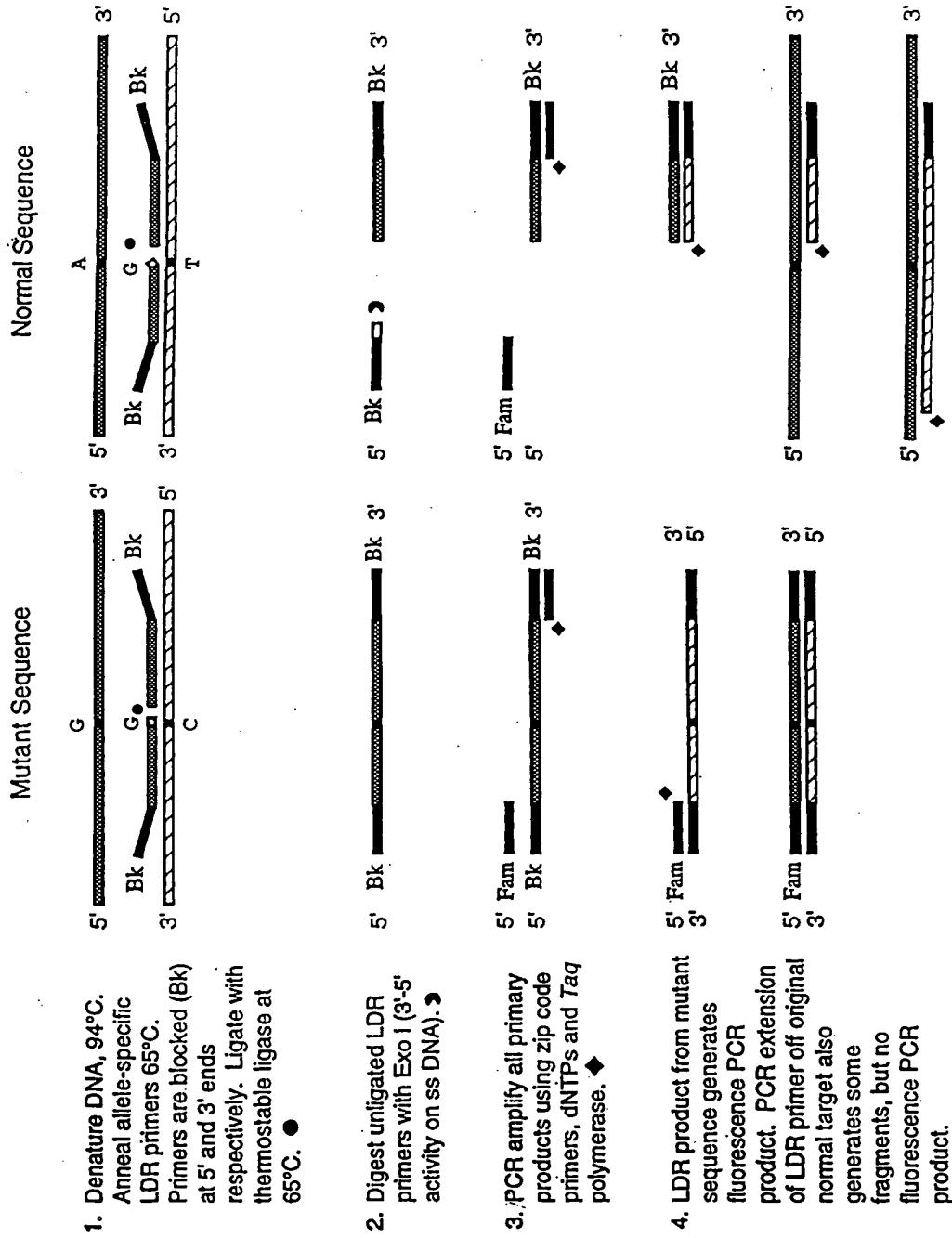
Allele specific LDR / PCR Problem



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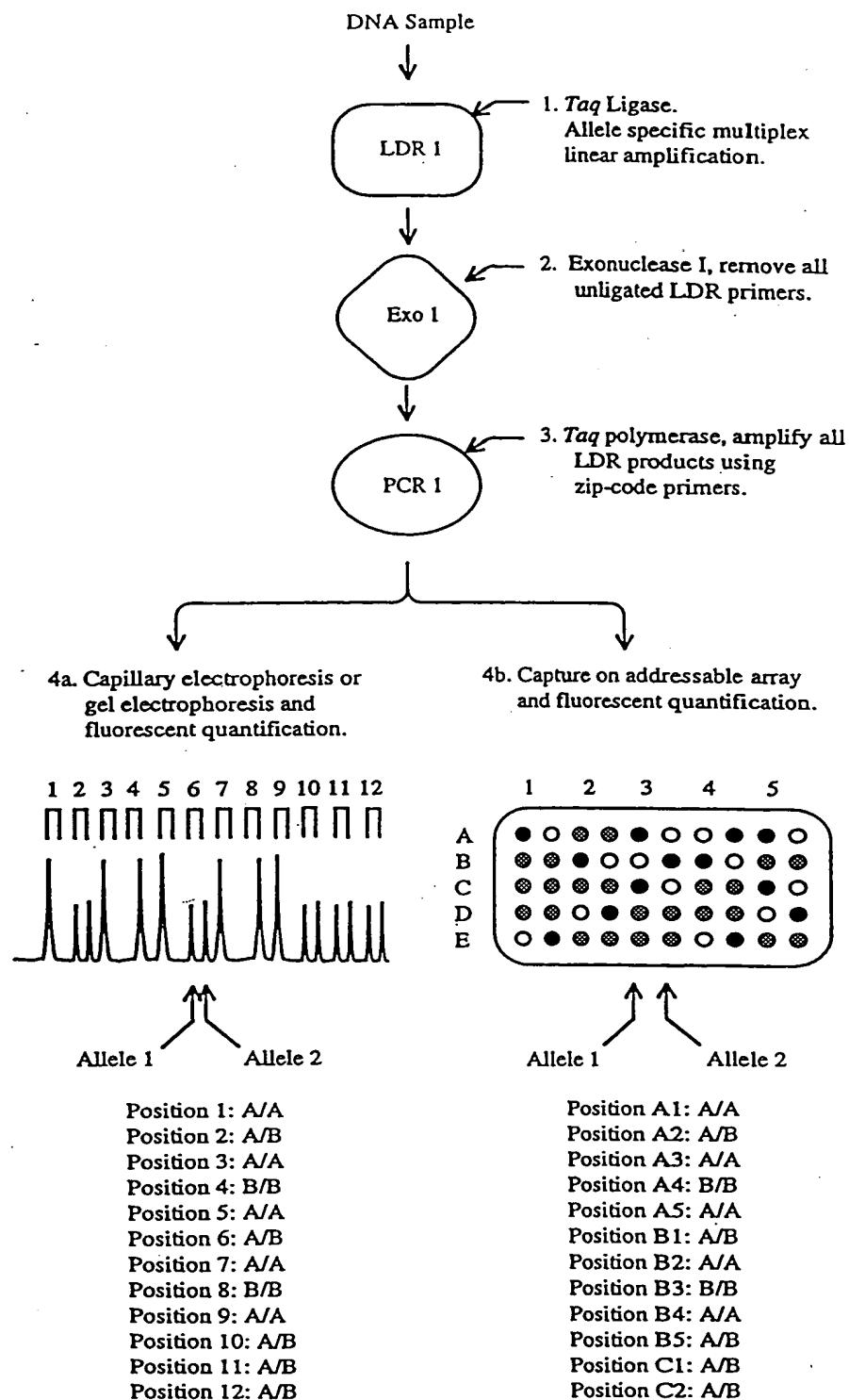
FIG. 11

Solution to allele specific LDR / PCR problem



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FIG. 12

**FIG. 13**

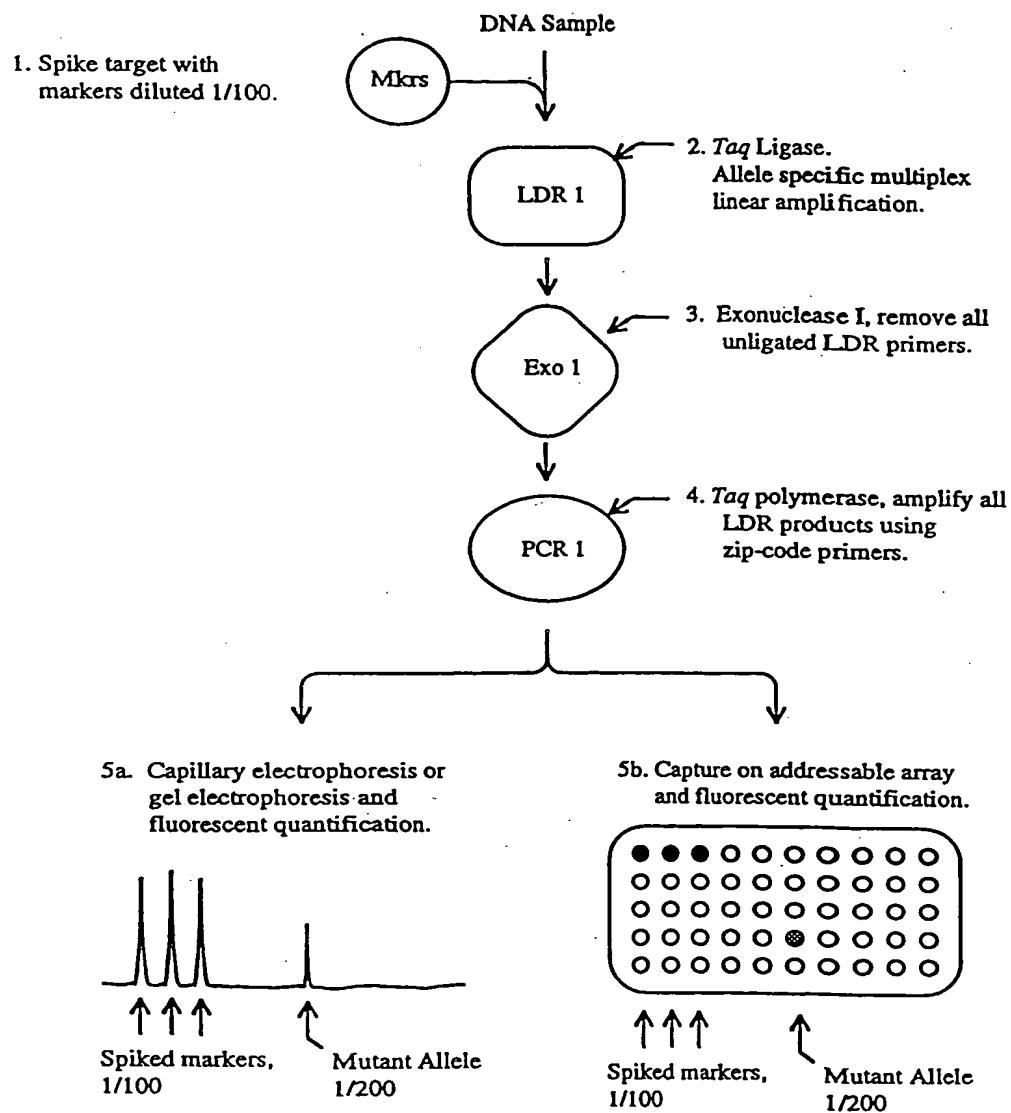
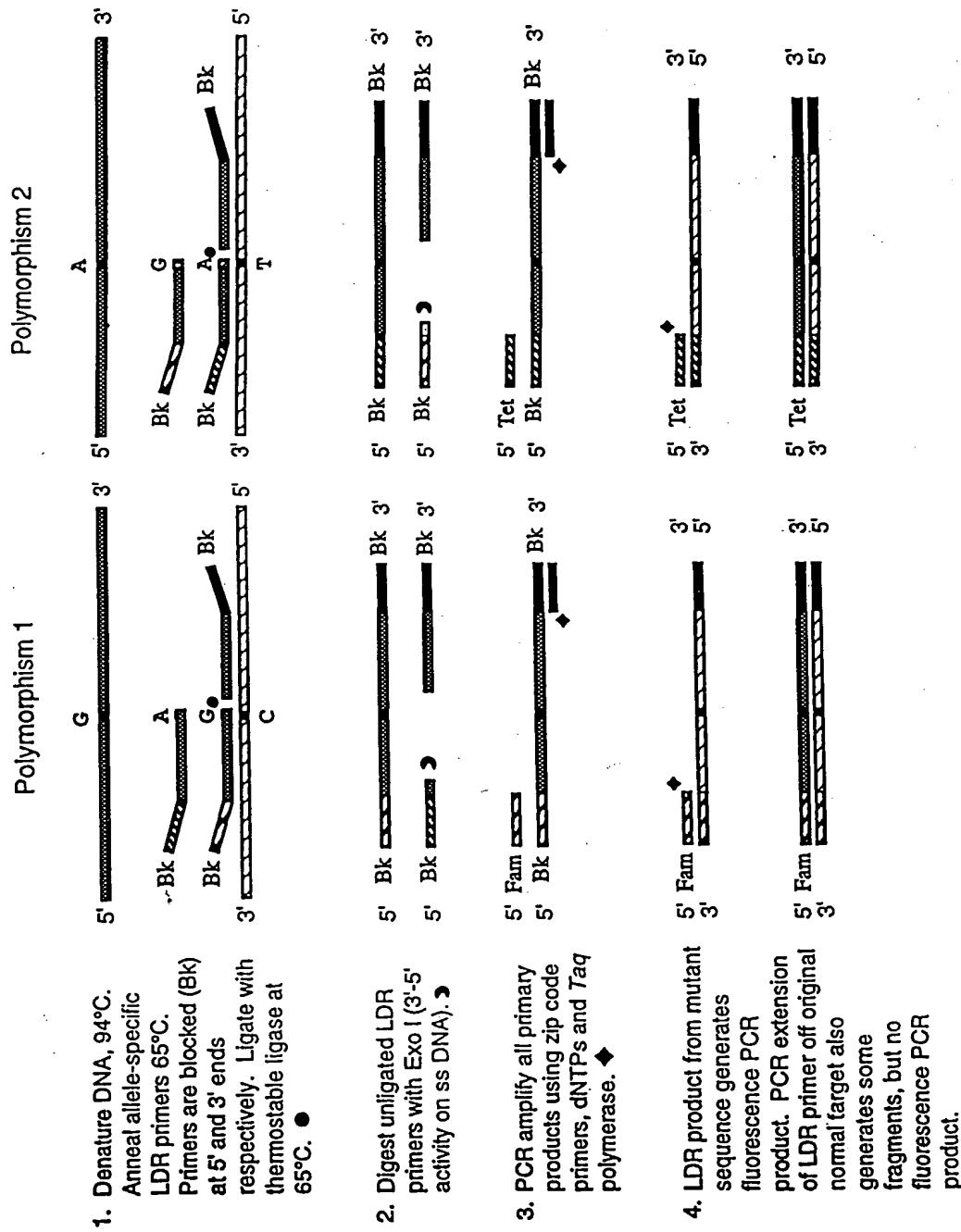


FIG. 14

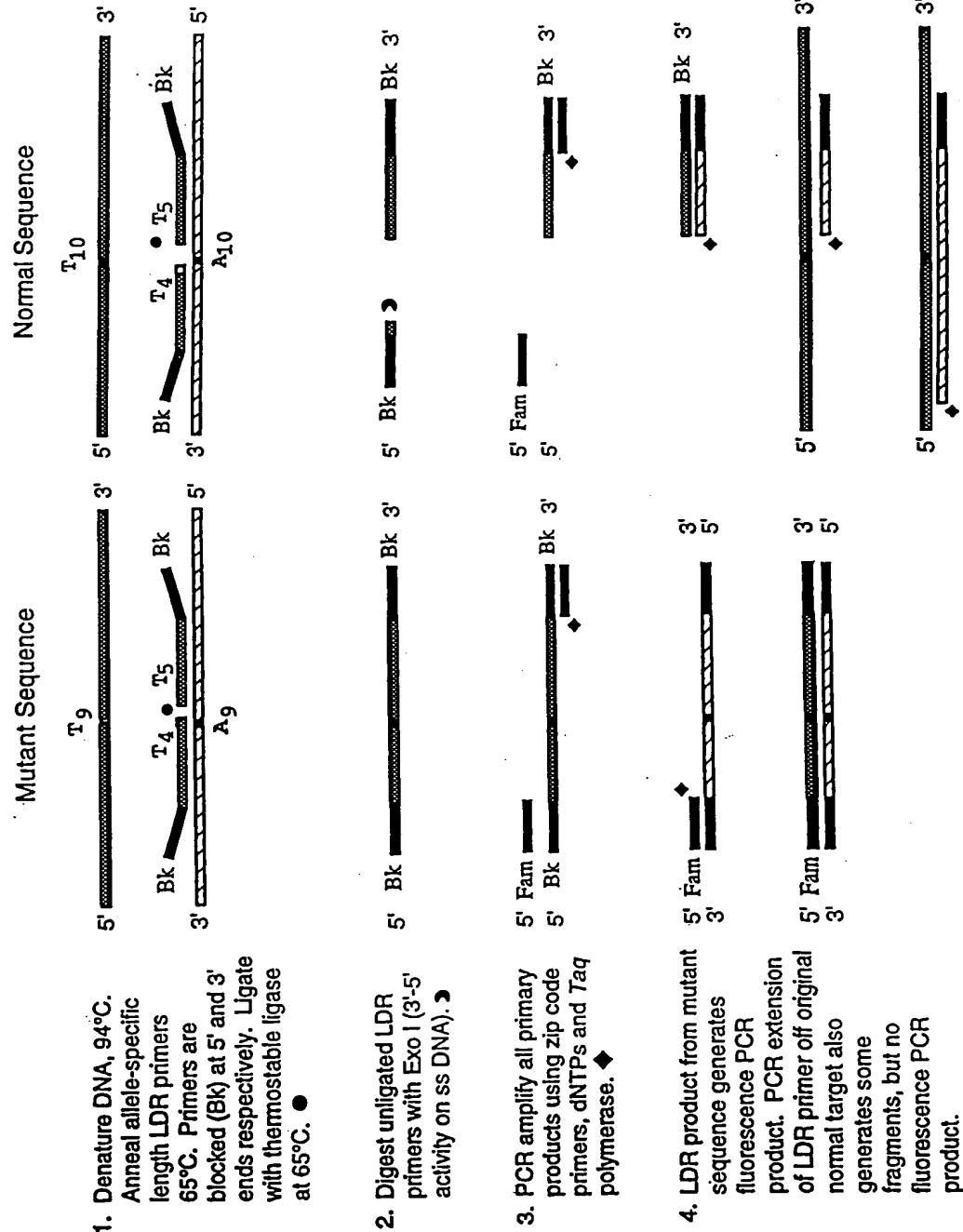
Allele specific LDR / PCR for mutations or polymorphisms



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FIG. 15

LDR / PCR of mononucleotide repeats using exonuclease selection



LDR / PCR of mononucleotide repeat polymorphisms using exonuclease selection

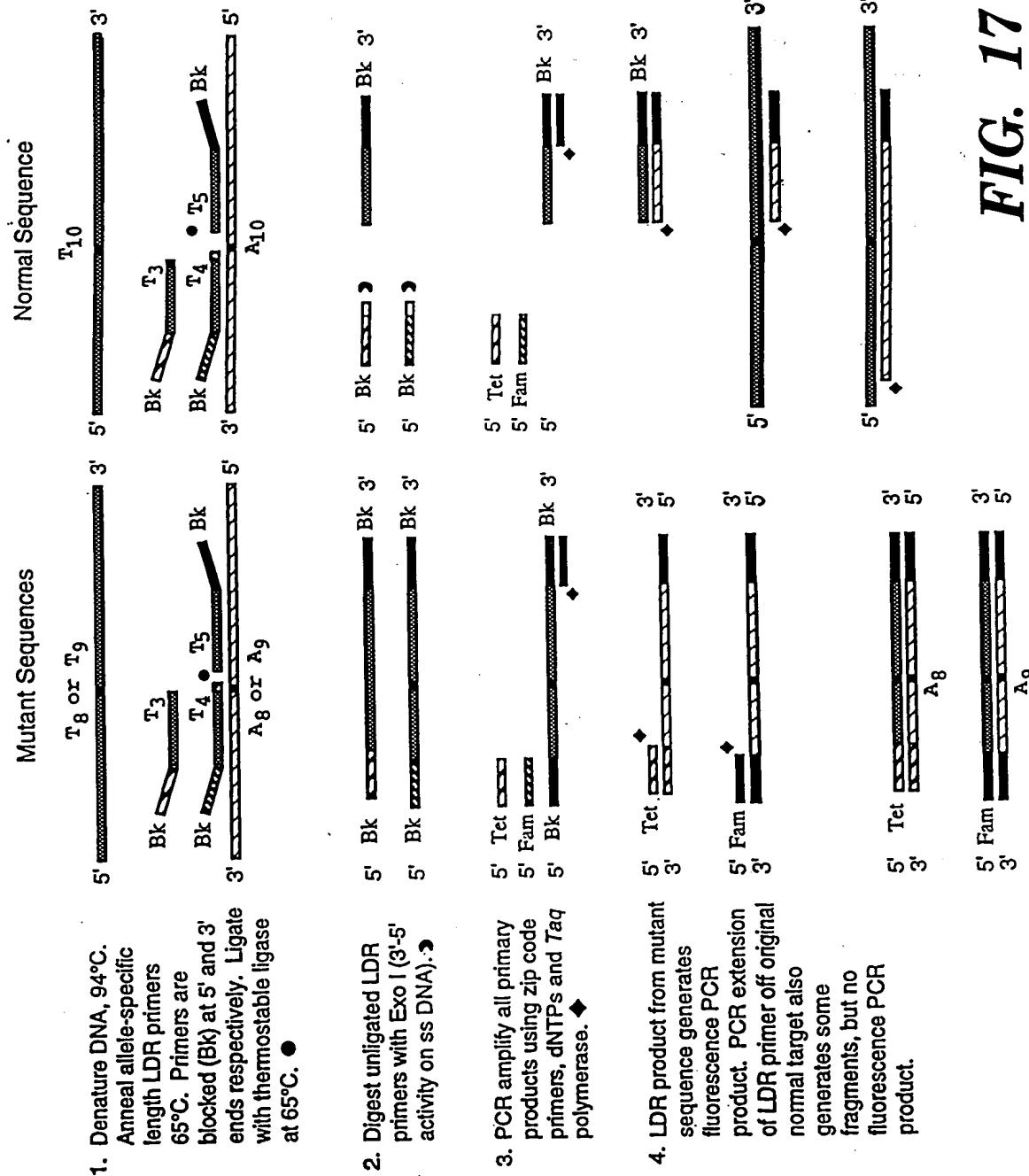


FIG. 17

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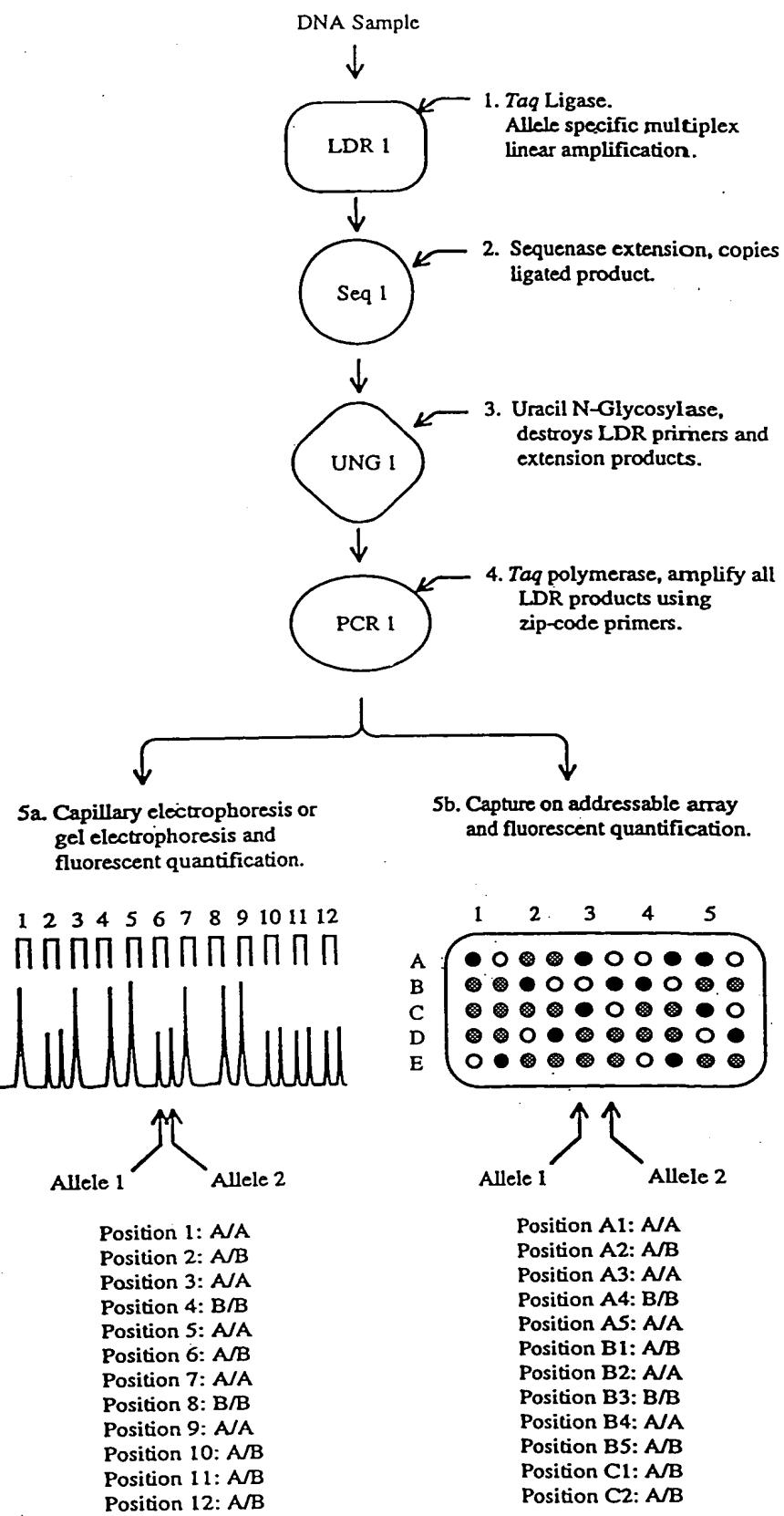
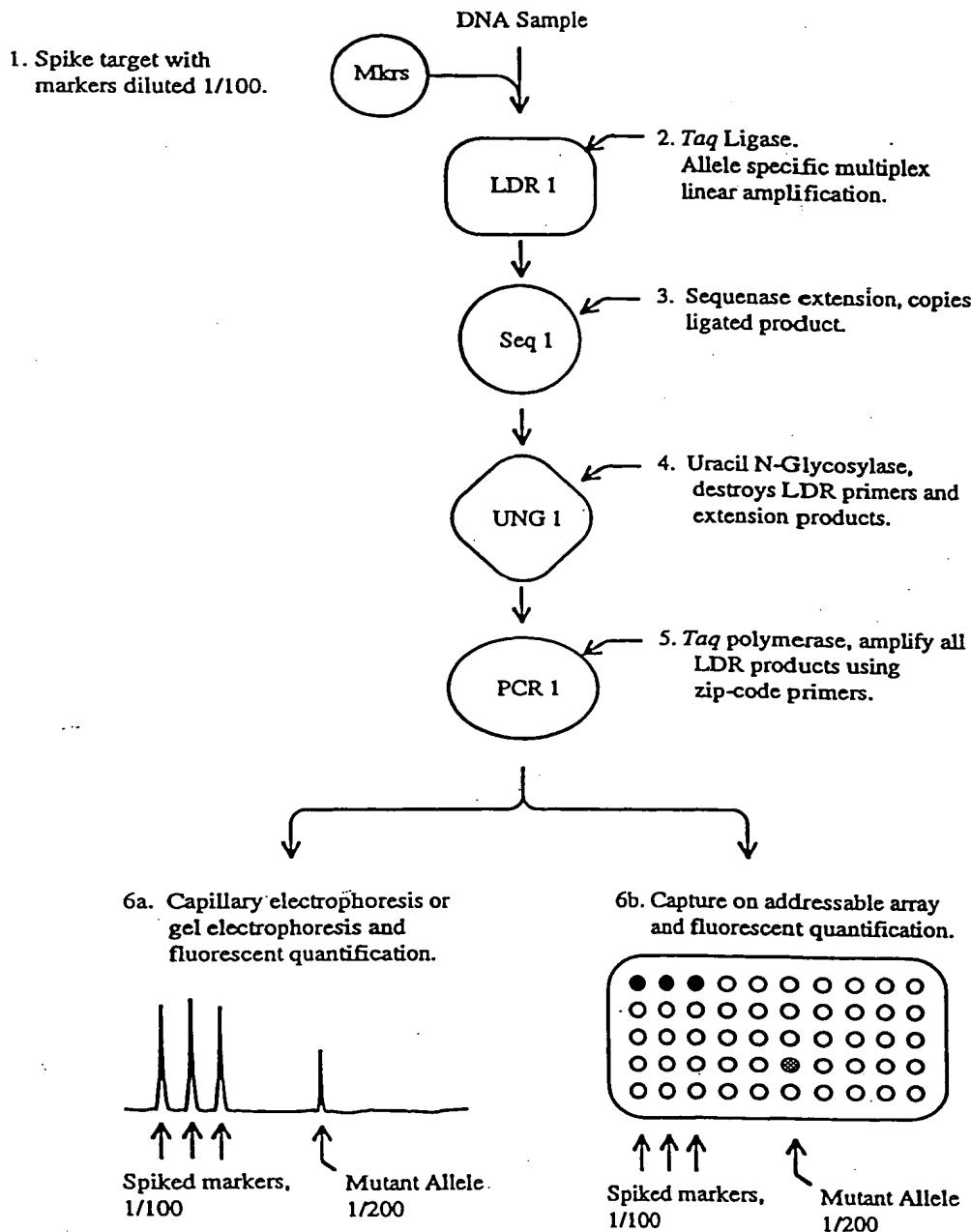
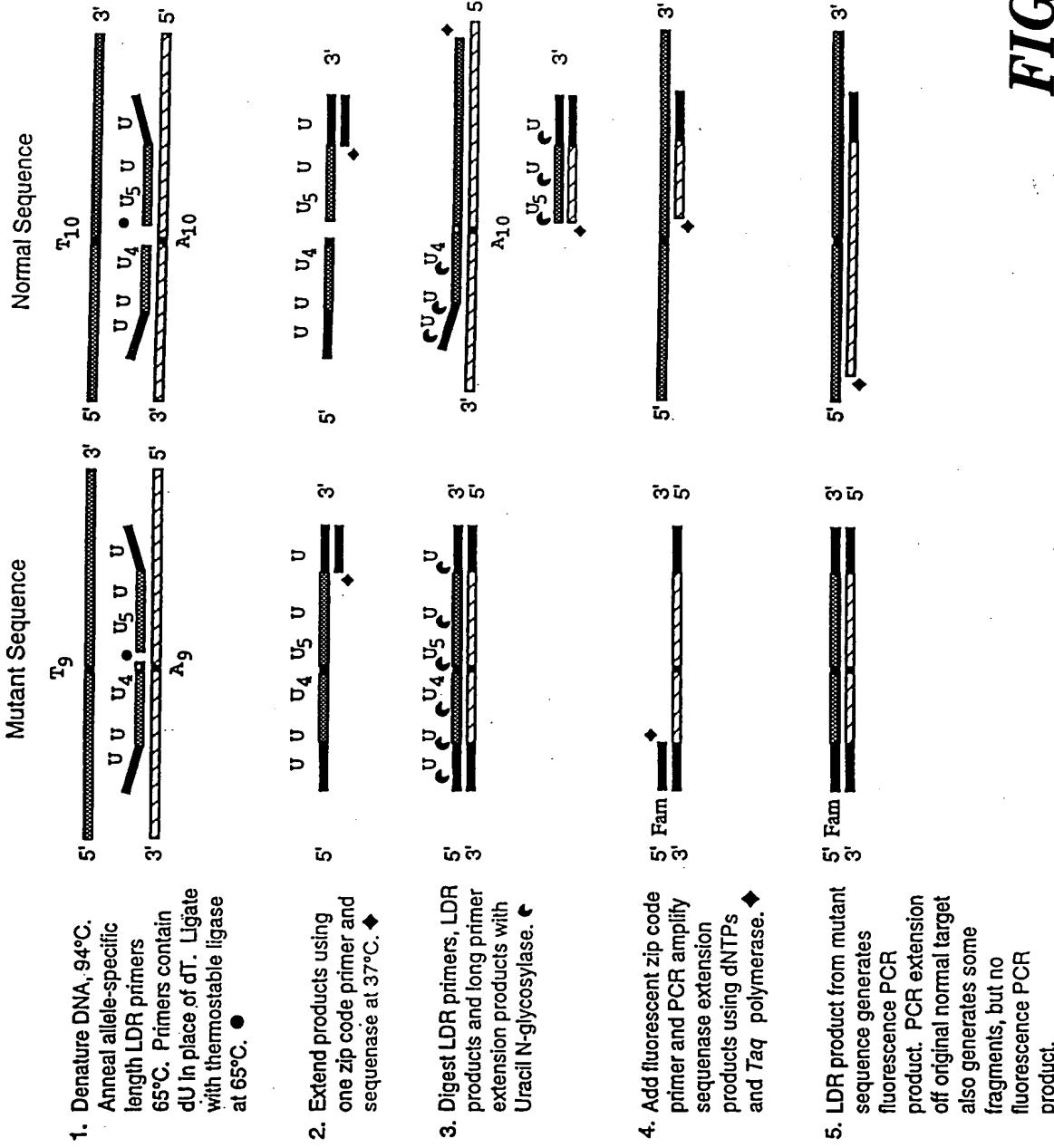


FIG. 18

**FIG. 19**

LDR / PCR of mononucleotide repeats using Uracil N-glycosylase selection



LDR / PCR of mononucleotide repeat polymorphisms using Uracil N-glycosylase selection

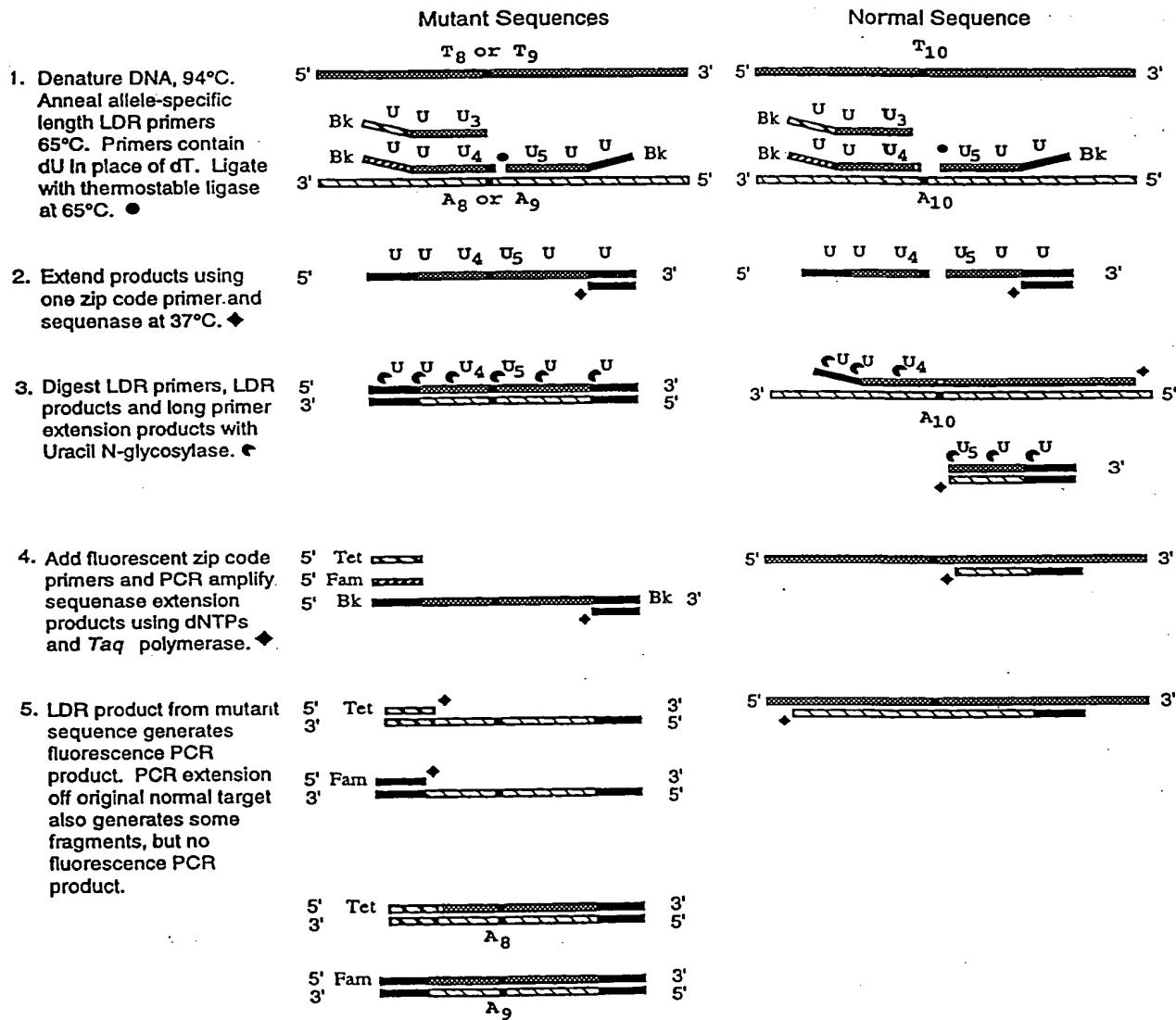


FIG. 21

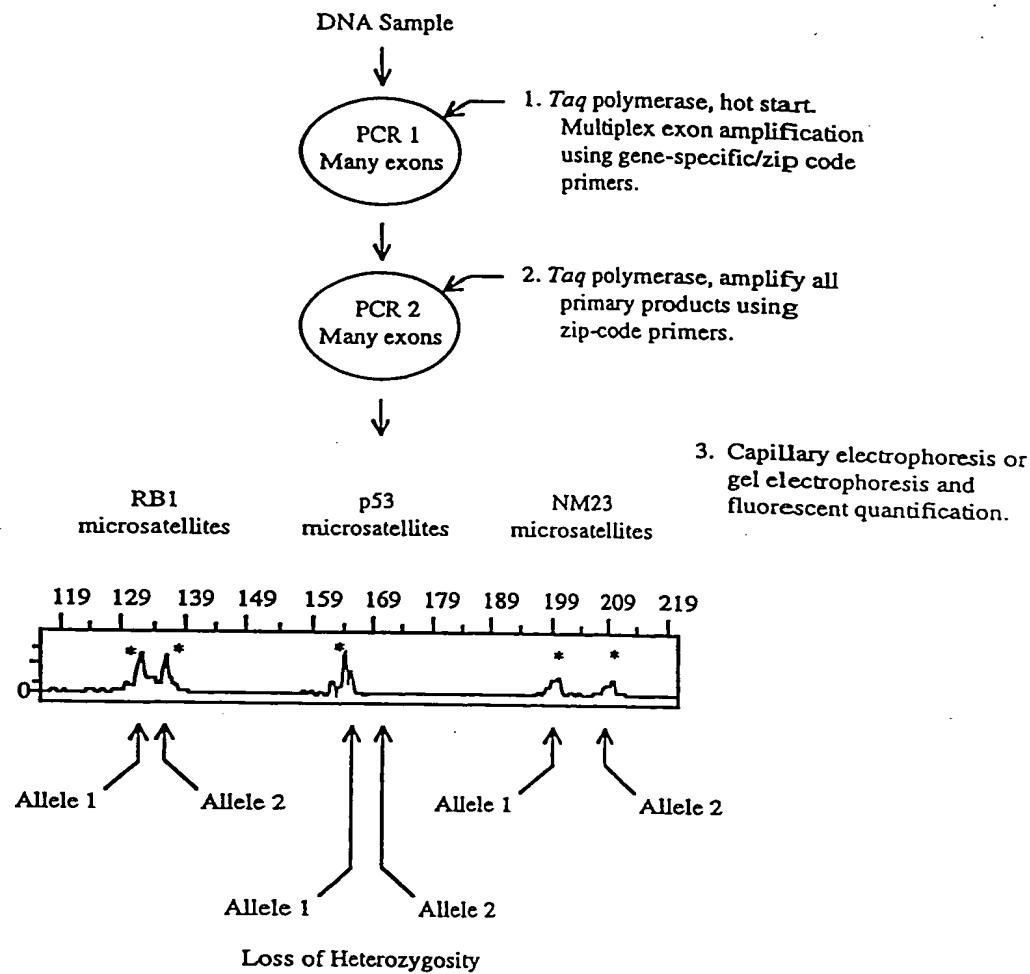
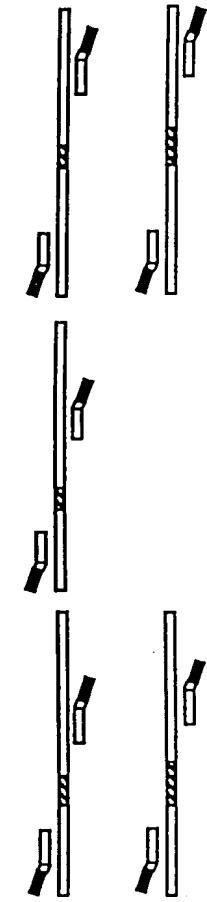


FIG. 22

PCR / PCR : Multiplex Microsatellite assays

Near RB1 gene
Chromosome 13q14.1

1. Denature DNA, 94°C.
Anneal longer
oligonucleotides, 65°C.
PCR amplify for 10 - 15
cycles.



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2. PCR amplify all primary products using *Taq* polymerase, dNTPs, and "zip code" primers-- one fluorescently labeled. Separate products by gel electrophoresis and determine loss of heterozygosity at informative loci.

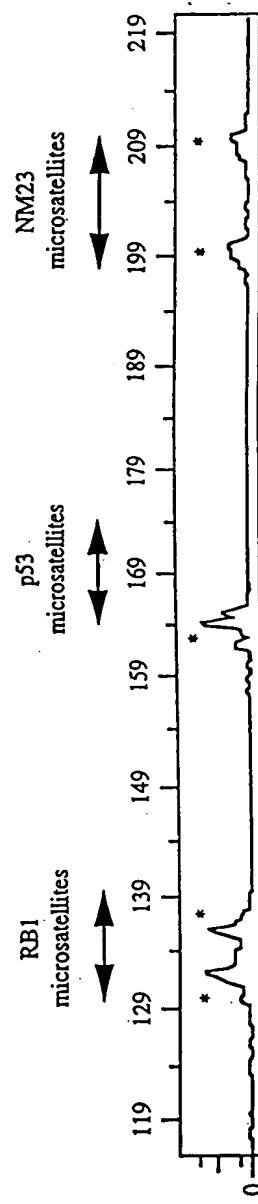


FIG. 23

Primer design for multiplex LDR / PCR

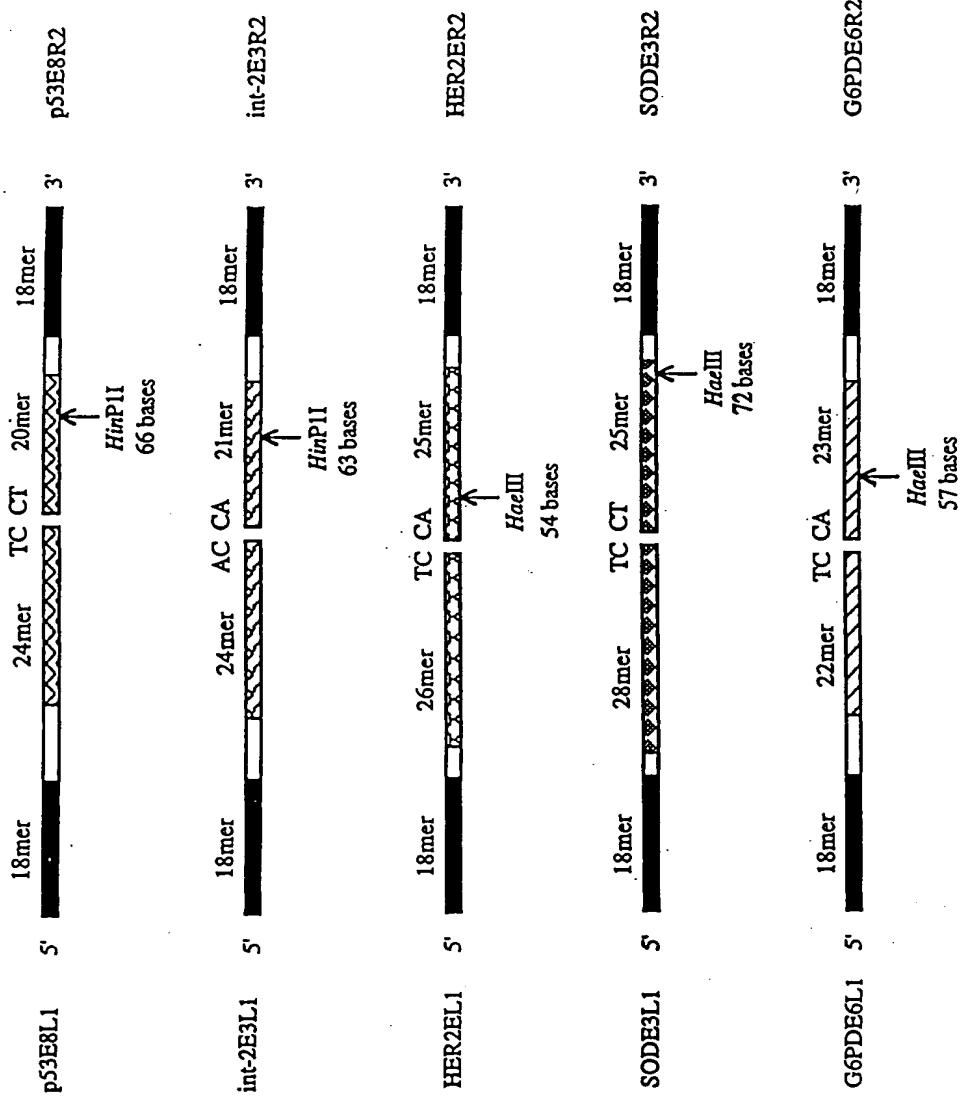


FIG. 24

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FIG. 25A

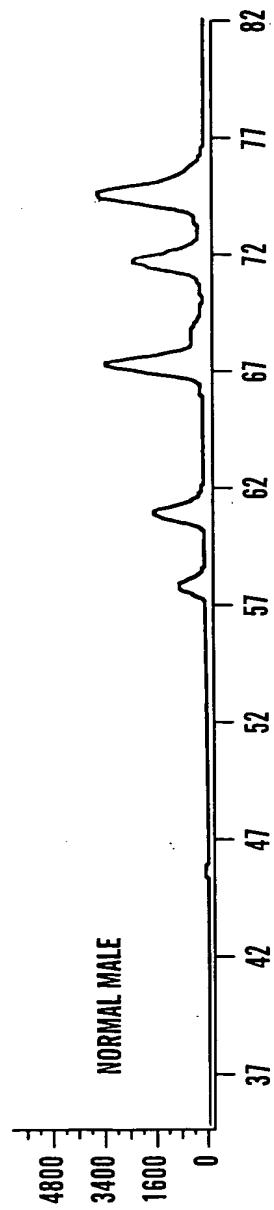


FIG. 25B

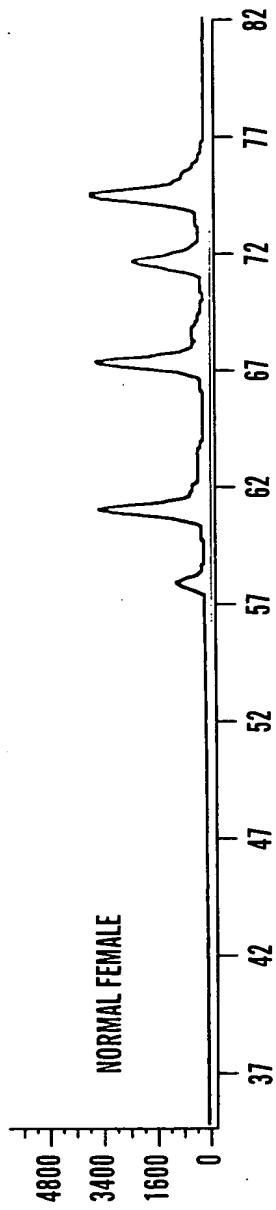


FIG. 25C

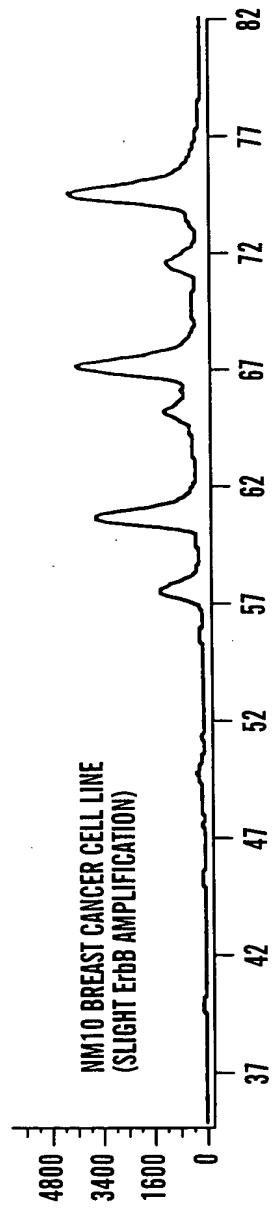


FIG. 25D

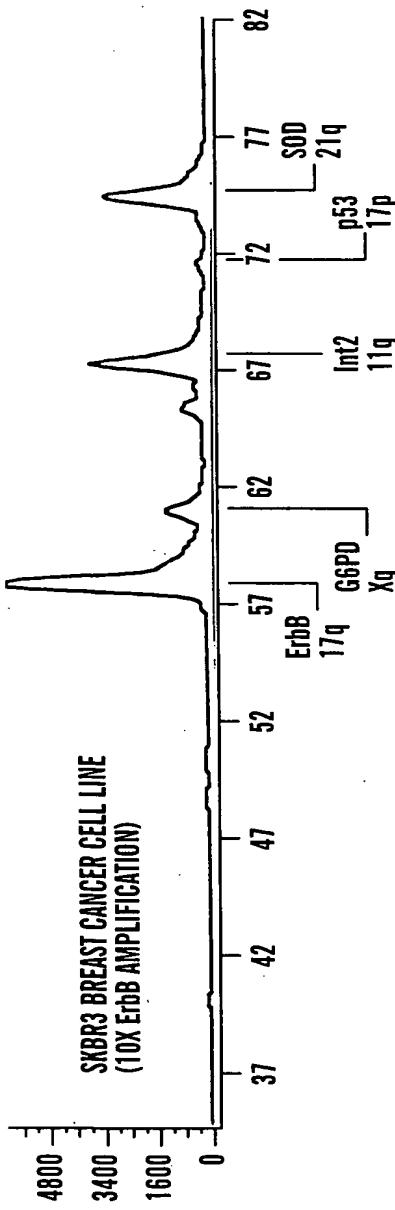


FIG. 26A

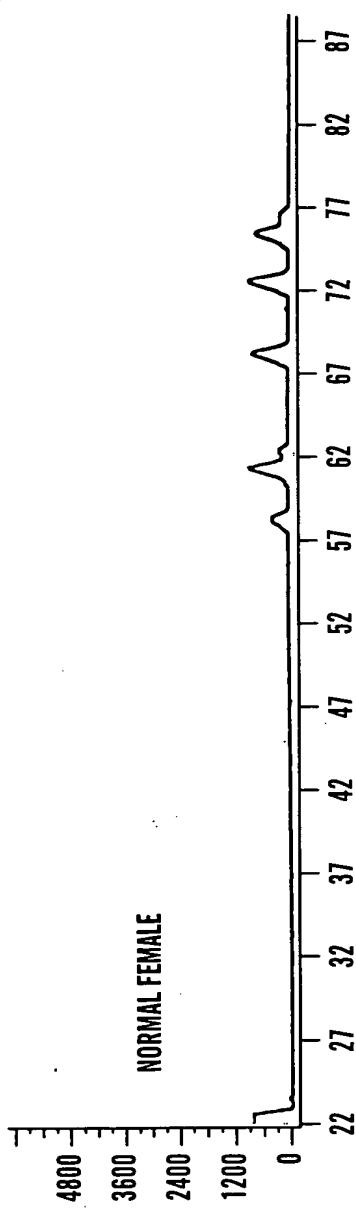


FIG. 26B

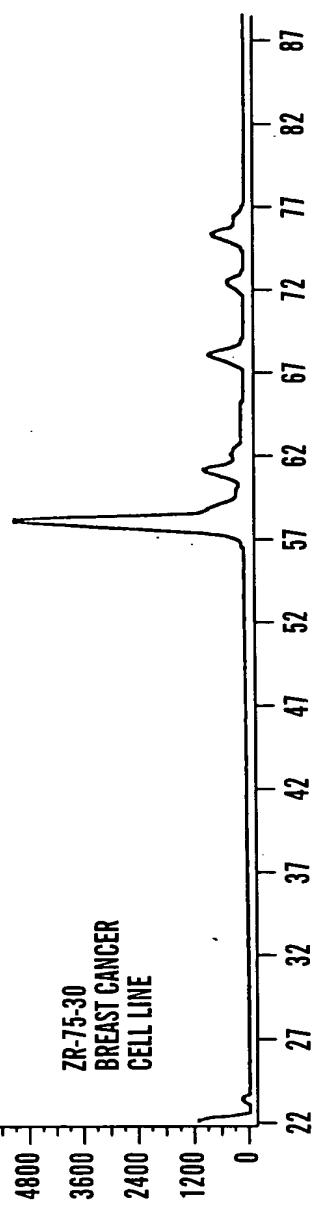
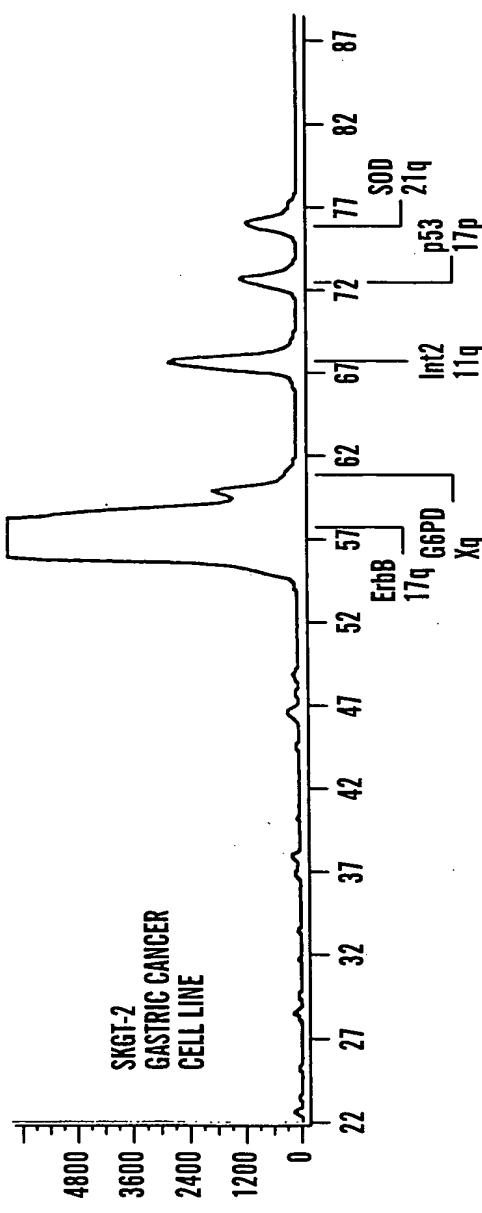


FIG. 26C



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FIG. 27A

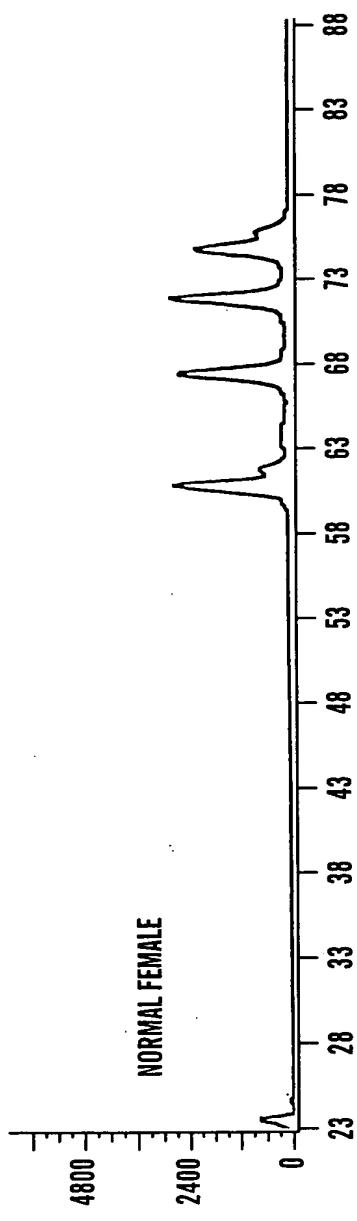


FIG. 27B

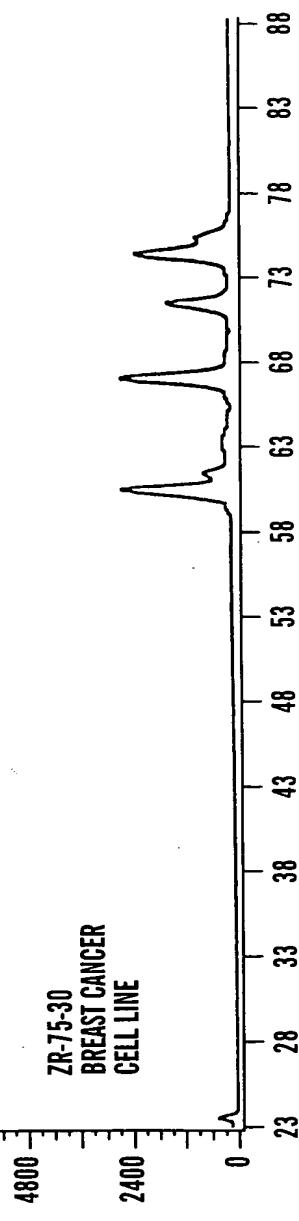
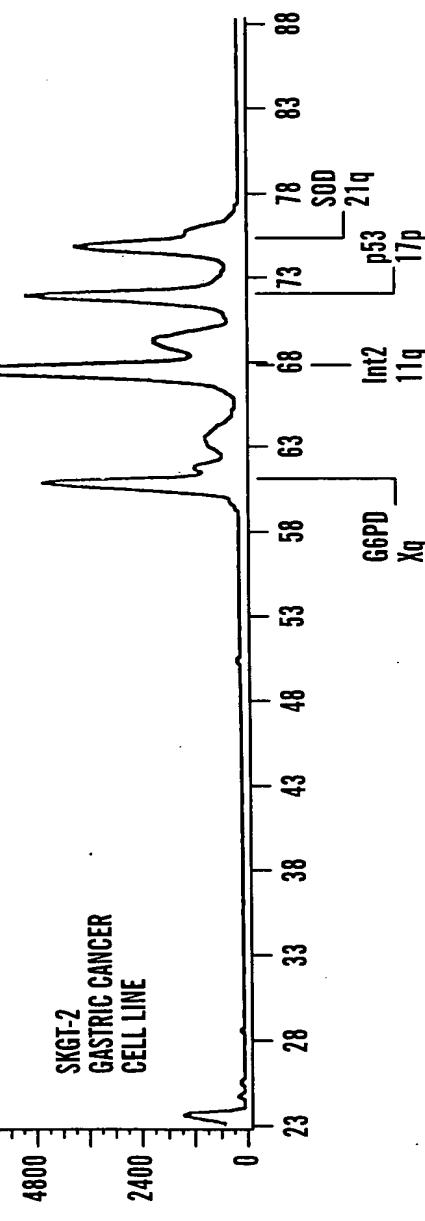


FIG. 27C



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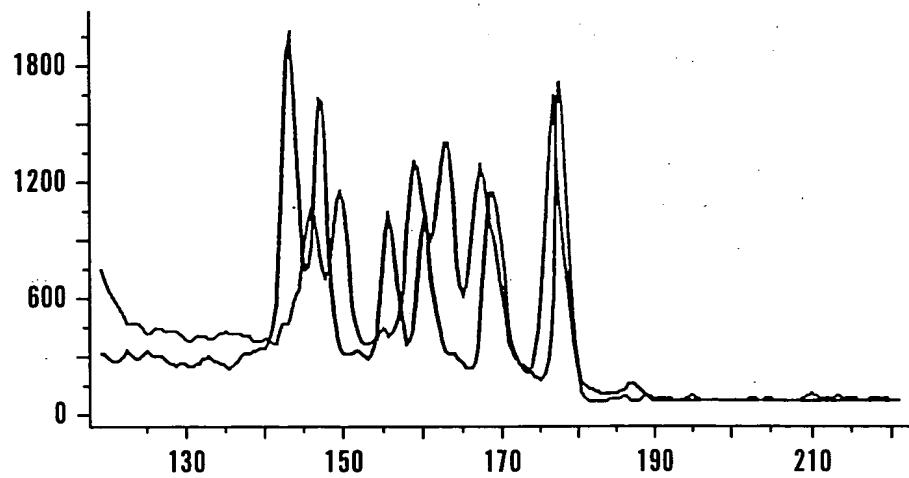


FIG. 28A

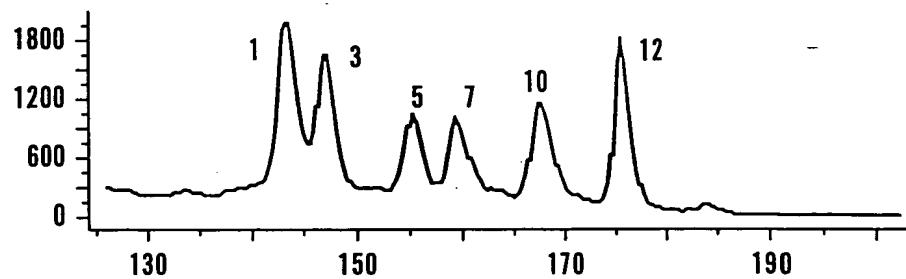


FIG. 28B

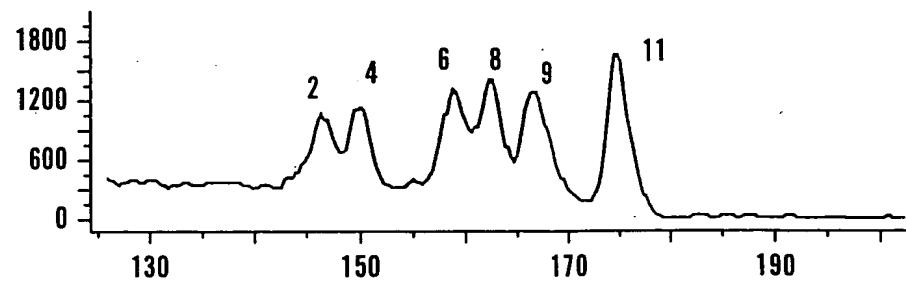


FIG. 28C

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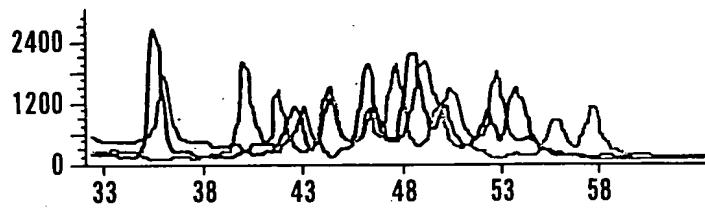


FIG. 29A

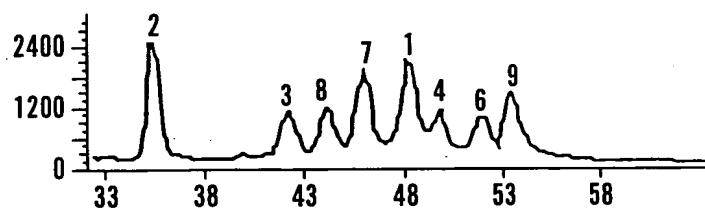


FIG. 29B

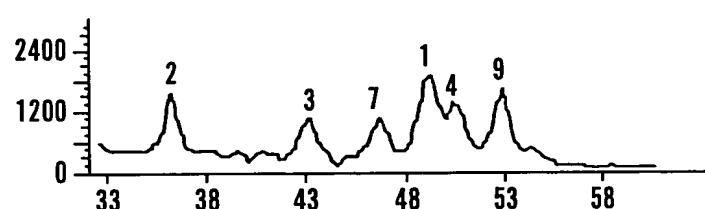


FIG. 29C

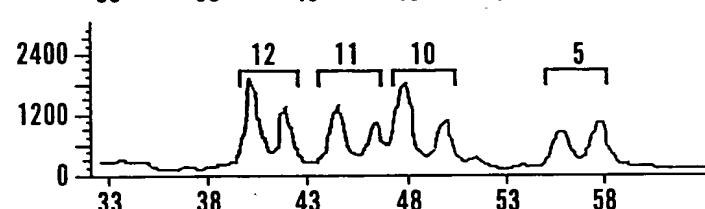


FIG. 29D

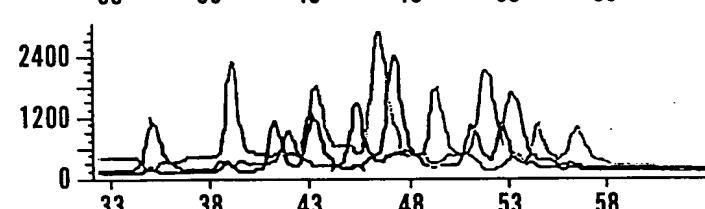


FIG. 29E

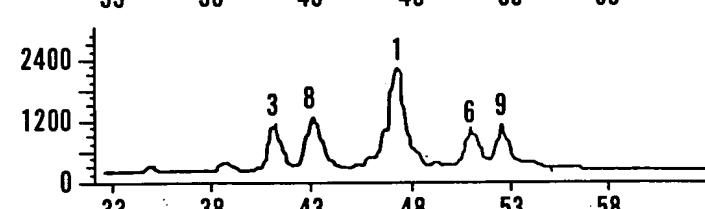


FIG. 29F

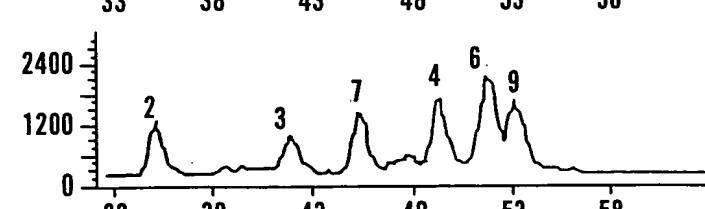


FIG. 29G

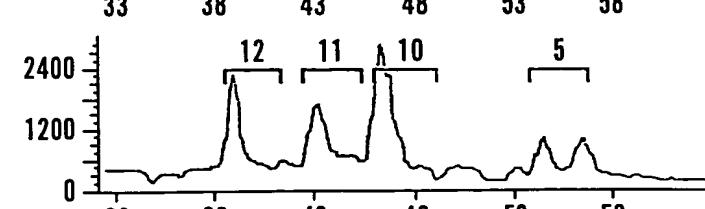


FIG. 29H